INTERACTIONS BETWEEN MOTONEURONES AND MUSCLES IN RESPECT OF THE CHARACTERISTIC SPEEDS OF THEIR RESPONSES

By A. J. BULLER*, J. C. ECCLES AND ROSAMOND M. ECCLES From the Department of Physiology, Australian National University, Canberra, Australia

(Received 1 September 1959)

In the preceding paper (Buller, Eccles & Eccles, 1960) evidence was presented which suggested that the differentiation of slow muscles of the cat hind limb to a large extent failed to occur after certain operative procedures on the spinal cord. This finding indicates that in some way the central nervous system controls muscle differentiation. A more analytic investigation into this postulated influence of nerve on muscle has been accomplished by dividing and cross-uniting nerves to fast and slow muscles, so that motoneurones formerly innervating the fast muscle come to innervate the slow muscle by virtue of the regenerative outgrowth of their fibres, and vice versa for the motoneurones of the slow muscle. The effect of this crossed innervation on the speed of muscle contraction has been tested at varying times after the cross-union. These investigations have also been carried out on animals subjected to the operative procedures on the spinal cord, as previously described (Buller et al. 1960). Cross-union experiments allow in addition an investigation into the possible effects in the reverse direction, i.e. of speed of muscle contraction on the conduction velocity of the axons and the after-hyperpolarization of the motoneurones which innervate it. A preliminary account of part of this investigation has already been published (Buller, Eccles & Eccles, 1958).

METHODS

Most of the cross-union operations have been performed on kittens 2–3 weeks old, but some operations had a wide age dispersion: on kittens as young as 6 days; on kittens from 3 weeks to 4 months; and on full-grown cats at least 6 months old. The operations were performed on anaesthetized animals with full aseptic precautions. Usually the cross-union has been effected between the nerves to soleus and flexor digitorum longus muscles (FDL), but there have also been cross-union experiments between the nerves to peroneal muscles and soleus and between the nerves to gracilis and crureus. The position of the nerve suture has been as far distal as possible from the locus of the nerve cross and as far as possible

^{*} Nuffield Travelling Fellows of the Royal Society. Present address: St Thomas's Hospital Medical School, London, S.E. 1.

from the muscle originally innervated by the nerve. This precaution was important in order to ensure that there was virtually full cross-regeneration uncontaminated by self-regeneration. However, in several of the early experiments a soleus nerve united to the peripheral stump of flexor digitorum longus nerve also achieved a small reinnervation of the medial side of soleus muscle. With the nerve pairs used it was possible to approximate the cut ends without appreciable traction. The respective proximal and distal stumps were then tied together by a very fine strand of nylon thread and the nerve sheaths were opened so that they would not impede the outgrowth of nerve fibres from the proximal stump into the distal stump. In about half the experiments the nerves were similarly divided on the control side, but each proximal stump was reunited to its own distal stump. The operations are illustrated diagrammatically in Fig. 1.

The investigations of muscle contractions were identical with those described in the previous paper (Buller et al. 1960). The nerves to the muscles were dissected out as far proximally as possible and cut centrally. It was particularly important to stimulate the cross-sutured nerves well proximal to the site of suture so that there could be no doubt that the muscle contraction was evoked by the motor fibres that originally supplied a particular muscle, e.g. that FDL motor nerve fibres were evoking a contraction in soleus muscle. No complications arise on account of regenerating afferent nerve fibres because they do not make effective motor connexions with muscle fibres (Gutmann, 1945, 1958; Weiss & Edds, 1945; Guth, 1956). In several experiments the conduction velocities of the various motor nerve fibres were measured by stimulating the muscle nerves and recording from the appropriate ventral roots, as described by Eccles, Eccles & Lundberg (1958). Finally, the time courses of after-hyperpolarizations of motoneurones were measured by the standard technique of intracellular recording (Eccles et al. 1958).

RESULTS

Nerve cross-union and muscle speeds

Comparison of Fig. 1B with A shows the invariable result of the crossunion operation: a considerable acceleration of the contraction of soleus muscle reinnervated by FDL nerve fibres, and, complementarily, a considerable slowing of the contraction of the FDL muscle reinnervated by soleus nerve fibres. The soleus and FDL in Fig. 1A and B had had an equivalent history of nerve degeneration and regeneration. The relative changes in contraction times must therefore arise on account of the nerve cross-union. There were comparable contraction times on the two sides of all the muscles with undisturbed innervation, only two being illustrated in Fig. 1. Not only was the contraction time of soleus shortened to 42 msec in Fig. 1A, as compared with the 60 msec for soleus after self-regeneration in Fig. 1B, but the time from summit to half-relaxation was correspondingly shortened to 45 from 62 msec. Complementarily, the FDL contraction times and half-relaxation times were lengthened from 33 to 59 msec and from 32 to 67 msec respectively. It will be seen that the two control muscles had twitch tensions within a few per cent of each other on the two sides. In contrast, the cross-innervated muscles developed only about half the twitch tension given by the corresponding self-reinnervated muscles on the control side.

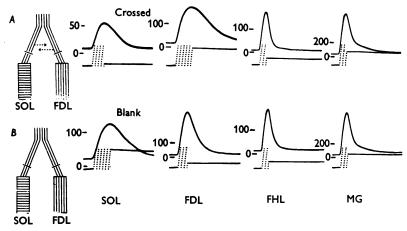


Fig. 1. Series of twitches for the muscles indicated by the symbols to show effect of nerve cross-union: SOL = soleus; FDL = flexor digitorum longus; FHL = flexor hallucis longus; MG = medial gastrocnemius. A shows the contractions for the left side in which the soleus and FDL nerves had been cross-sutured (see diagram to left) at the age of 21 days, the experimental investigation being 30 days later. In the control side, B, the soleus and FDL had been severed and rejoined at the original operation, as in diagram to left. The decade-counter device (lower beam) gives the contraction times in milliseconds. Weight of kitten = $0.85 \, \mathrm{kg}$. Tension scales in grams indicate zero tension.

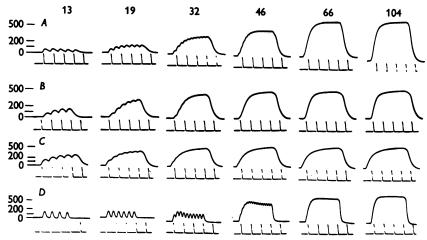


Fig. 2. Repetitive muscle responses to show effect of nerve cross-union. The original nerve operations were performed, as in the diagrams of Fig. 1, on the 16-day-old kitten, and 60 days later the repetitive muscle responses at the indicated frequencies were recorded for the soleus and FDL muscles on the two sides. In the A series the soleus muscle was activated by the FDL nerve, and in the B series by the soleus nerve, as for soleus in Fig. 1A and B respectively. Likewise in the C series FDL muscle was activated by the soleus nerve, and in the D series by the FDL nerve, as for FDL muscle in Fig. 1A and B respectively. Weight of kitten = 1·4 kg. Note tension scales in grams and time scales in 100 msec.

As would be expected, there was an equivalent change in the summation of contractions produced by repetitive stimulation (Fig. 2). The soleus innervated by FDL motoneurones (A) required a considerably higher frequency of stimulation to give an equivalent fusion of contractions than in B, and conversely with the repetitive responses (C) of the FDL muscle innervated by soleus motoneurones. Plotting of the frequency-response curves (cf. Buller et al. 1960, Fig. 5F-J) shows that in Fig. 2 the stimulus interval for a contraction tension midway between the twitch and the maximum tetanus was shortened from 57 to 28 msec for the FDL-innervated soleus muscle and lengthened from 25 to 65 msec for the soleus-innervated FDL.

It has been regularly observed (cf. Fig. 2) that the ratio of maximum tetanic tension to twitch tension was abnormally high for soleus muscles reinnervated by FDL nerves. The values ranged from 7 to 16, as against normal values of 3 to 8 for self-reinnervated soleus muscles. In contrast, the ratios were in the range 2·5-4·5 for FDL muscles with either self-or cross-reinnervation, which is the range normally observed for both fast and slow muscles of the hind limb (cf. Cooper & Eccles, 1930). However, gracilis always gave exceptionally high ratios, usually 5-7, presumably on account of the summation of the extremely fast component (cf. Buller et al. 1960).

The plotted points (open circles) in Fig. 3A, B give the contraction times of FDL and soleus muscles for the whole series of experiments in which the nerve cross-union was performed in kittens 16-22 days old, with control self-reinnervation on the other side (filled circles). Thus in each case the arrow gives the change in contraction time attributable to the alien innervation. Invariably, soleus reinnervation substantially slowed the FDL muscle, whereas FDL reinnervation quickened soleus muscle. In Fig. 3A, B there is no indication of a trend in the magnitude of the change with increasing duration of the alien innervation, there being as large a change at 30 days post-operatively as at 90-100 days. However, the series is distorted by the values at 10-16 weeks from three kittens of the same litter that had abnormally fast soleus muscles. Moreover, further investigation is required at shorter intervals post-operatively. So far 23 days has been the briefest post-operative test period (crosses through circles in Fig. 3A, B), and even then the changes appeared to be as fully developed as with longer test periods.

In general, the plotted points in Fig. 3A, B show that the effect of the alien innervation falls short of a complete transformation of the muscle, the transformed FDL being faster than soleus muscle at an equivalent age, as indicated by the broken line in A; while the transformed soleus likewise remains a little slower than a fast muscle, as indicated by the broken line in B. One possible explanation of this incomplete transformation would be that the operation was performed on kittens 16-22 days old when there had already been a considerable differentiation into the fast and slow types

(cf. Buller $et\ al.$ 1960). However, two further investigations eliminated this explanation: the transformation was similarly incomplete when cross-union was performed on 6-day-old kittens (Fig. 4); and there was just as effective a transformation when the cross-union was performed on full-grown cats (Fig. 5). The plotted points to the extreme right of Fig. 3C, D are the means for the three experiments in which the cross-unions were performed on full-grown cats (over 6 months). With all the experiments plotted in Fig. 3C, D there was no control operation on the other side. With all the points for ages in excess of 16 weeks the post-operative test periods were very prolonged (100–200 days), but even then the transformations were in general incomplete.

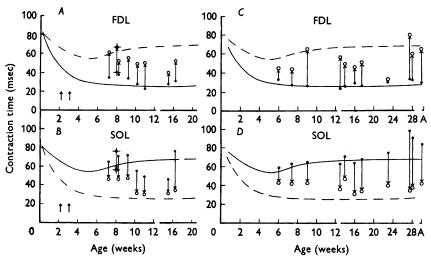


Fig. 3. Plotting of muscle contraction times to show changes produced in all experiments on cross-union between soleus and FDL nerves. Ordinate, muscle contraction times from records such as those of Fig. 1 plotted against abscissa, age in weeks. A and B give contraction times for FDL and soleus muscles for kittens that were operated at 16–22 days old (age range indicated by arrows), the open circles and filled circles giving respectively the contraction times for the alien-innervated and control muscles as in the diagrams to the left of Fig. 1. The arrows give the direction and magnitude of the change produced by the cross-union. The standard curves for contraction time—age (Buller et al. 1960, Fig. 3) are shown as a continuous line for the muscle itself and a broken line for the muscle belonging to the nerve that now provides its innervation; note that the direction of the change is always towards the curve characteristic of the new innervation. The crosses distinguish the points of the 8-week old animal, because that cross-union operation was performed on an older animal ($4\frac{1}{2}$ weeks) than the others.

In C and D the open circles have a similar significance as in A and B, but the filled circles give contraction times for muscles on which no control operation had been performed, i.e. on muscles that had retained their normal innervation throughout. The points plotted for the adult (marked A on abscissae) are the means of three experiments, one of which is illustrated in Fig. 5.

It is evident enough from the muscle twitches illustrated in Figs. 1, 4, 5 that the cross-union brings about a change in the relaxation time as well as in the contraction time. This is illustrated in Fig. 6A, B, where times for half-relaxation have been plotted with the same conventions as for the contraction times in Fig. 3. There has been a pooling in Fig. 6A, B of the measurements for all experiments plotted in Fig. 3A-D, and the standard

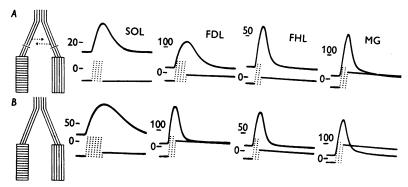


Fig. 4. A. Series of muscle twitches as in Fig. 1A, but the operation was performed on a 6-day-old kitten and the experimental observation was made 45 days later. As shown in diagram to the left, there was no control operation on the other side, i.e. the B series of records are for muscles with undisturbed innervation. Weight of kitten = 0.57 kg. Tension scale in grams.

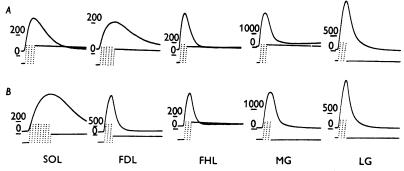


Fig. 5. As in Fig. 1, but the initial cross-union operation was performed on an adult cat; and as in Fig. 4 there was no control operation on the other side. The final experimental test was made 201 days after the operation. Weight of cat = 2.8 kg. Tension scale in grams.

curves for normal muscles (cf. Buller $et\ al.$ 1960, Fig. 4) have been drawn as in Fig. 3. As indicated by the arrows, there has been with only one exception a considerable transformation of all muscles with alien nerve supply, but again the transformation has been incomplete. The very small lengthening observed for FDL muscle at the 23-week experiment (cf. Fig. 3C) remains unexplained.

The repetitive responses illustrated in Fig. 2 reveal that the transformation also obtains for the tetanic responses of muscle. This is illustrated in Fig. 6C, D for all the cross-innervated muscles that were tetanically stimulated. As has been described previously (Buller et al. 1960), the time factor for tetanic summation has been measured as the stimulus interval at which the tetanic tension lies midway between the twitch and maximum tetanic tensions. By this criterion, there has been a considerable transformation of all the cross-innervated muscles, but again it tended to be incomplete.

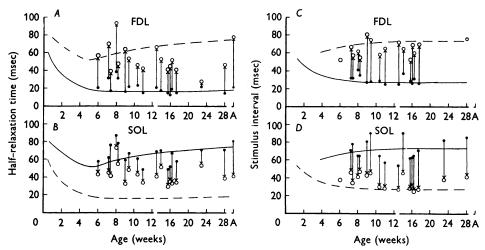


Fig. 6. Plotting as in Fig. 3, but for other measurements on muscle contraction to show effects of nerve cross-union. Half-relaxation times of the muscle twitches corresponding to the two series of Figs. 3A, B and C, D are pooled in Fig. 6A, B. The standard curves, plotted as continuous and broken lines as in Fig. 3, are those determined for half-relaxation times of normal muscles (Buller $et\ al.$ 1960, fig. 4). In C and D there are plotted as ordinates the stimulus intervals at which repetitive stimulation gave half of the full tetanic summation. Again the standard curves are those determined for the normal muscles (Buller $et\ al.$ 1959, fig. 6).

A possible explanation of the slowing of the FDL contraction when reinnervated by soleus nerve fibres (Figs. 1, 4, 5) would be that impulses in the regenerated soleus nerve fibres were evoking repetitive discharges from the FDL motor end-plates. Single nerve volleys evoke repetitive discharges from motor end-plates in which the cholinesterase has been inactivated (Brown, Dale & Feldberg, 1936; Brown, 1937), and even from the normal motor end-plates in the unanaesthetized preparation (Eccles & O'Connor, 1938; Feng, Li & Ting, 1939), but such repetitive discharges have not been observed in preparations anaesthetized as in the present investigations (Eccles & O'Connor, 1939). In addition, there are two reasons why repetitive discharges cannot account for the lengthening of the FDL contractions

in Fig. 1A: the muscle action potential showed no trace of repetitive discharge (Fig. 7B); when neuromuscular transmission was blocked by curare, the muscle twitches evoked by direct stimulation of the muscle fibres showed a similar difference when compared with the twitches of the control muscle. For example, in Fig. 7G the FDL twitch was throughout much longer than the control FHL twitch (Fig. 7F) of the same curarized preparation, 45-27 msec for the respective contraction times and 49-17 msec for the half-relaxation times, which were virtually the same ratios as before the curarization (Fig. 7C, A). The responses to repetitive stimulation (Fig. 2C) further show that the prolongation of the FDL twitches

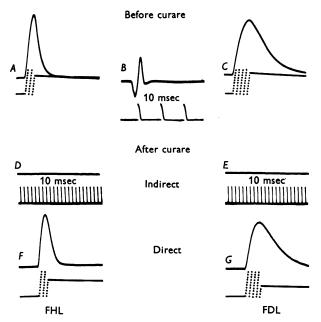


Fig. 7. A and C are twitches of FHL and FDL muscles with contraction times of 27 and 48 msec respectively, the FDL being slowed by cross-union with soleus nerve. B is the action potential led from the surface of FDL muscle. D, E show that after intravenous administration of curare nerve stimulation failed with both muscles; but direct stimulation was effective in evoking twitches, F, G, comparable with A, C. Weight of cat E E0 kg.

was not due to repetitive end-plate discharge, for the prolongation was just as prominent with repetitive stimulation, which is contrary to the observations on repetitive discharge from unanaesthetized motor end-plates (Eccles & O'Connor, 1938; Feng et al. 1939).

Since the prolonged twitches of FDL muscle fibres innervated by the regenerated soleus nerve were observed even with direct stimulation of the curarized muscle, it must be concluded that the contraction mechanism of

the muscle has been slowed. It must likewise be concluded that the contraction mechanism of the soleus muscle has been quickened on account of its regenerated innervation from FDL motoneurones.

There has been sufficient investigation with other nerve-crossing experiments to allow generalization of the observations with soleus-FDL crossing to the crossing of any combination of nerves to fast and slow muscles. For example, Fig. 8B shows the slowing of twitches of gracilis muscle (contraction times lengthened from 26 to 56 msec) when reinnervated by the slow crureus motoneurones, while in Fig. 8A the contraction time of the crureus muscle was shortened from 78 to 37 msec when it was reinnervated by the fast gracilis motoneurones. Similarly the twitch of soleus muscle was shortened from 94 to 36 msec when it was reinnervated by motoneurones belonging to the fast peronei muscles (Fig. 8C). Thus in general it

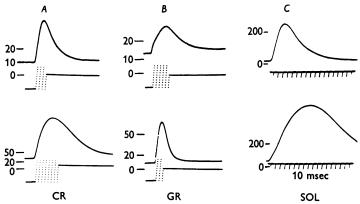


Fig. 8. A, B, twitch contractions of gracilis and crureus muscles cross-innervated from crureus and gracilis nerves respectively in the upper record; below, the normally innervated control muscles on the other side. The operation was performed on the 22-day-old kitten, and the subsequent experimental test 42 days later; weight of kitten = 0.63 kg. C, twitch contractions of soleus muscle, the upper record 180 days after cross-union with the nerve to the peronei muscles (operation on 19-day-old kitten), the lower record being the control from the other side with unmolested innervation. Weight of cat = 2.4 kg.

can be concluded that the regenerated nerve fibres change the speed of the muscle contraction so as to bring it into an appropriate relationship with the frequency characteristics of the innervating motoneurones (cf. Eccles et al. 1958). The cross-union experiments further establish that this neural influence is continuously exerted even in the adult. For example, experiments such as that illustrated in Fig. 5 show that the adult soleus motoneurones are very effective in slowing the speed of a fast muscle which they reinnervate, and at the same time the soleus muscle deprived of their influence becomes a fast muscle.

The neural influence is sharply restricted to the muscle fibres that receive the alien reinnervation. For example, in Fig. 9A the twitch contraction time of that part of the crureus muscle supplied by a branch from the vastus medialis nerve has been shortened from 77 to 30 msec by reinnervation from the fast gracilis motoneurones. In contrast there was no significant difference for that part of the same crureus muscle with unchanged innervation by the branch from vastus lateralis nerve (Fig. 9B). A similar experiment is illustrated in Fig. 9C-E, where in C the FDL muscle with alien innervation from soleus nerve had a twitch contraction time of 72 msec in contrast to the value of 34 msec for that part with its normal innervation. There was a simple arithmetical summation of the two contractions when evoked in sufficiently close temporal sequence (Fig. 9D), as may be seen by comparison with Fig. 9E, where comparable wave forms were produced by an electrical model that was designed for arithmetical summation.

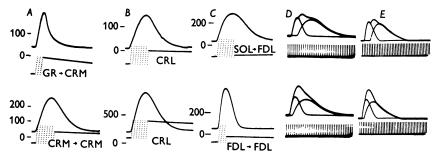


Fig. 9. A, B, twitch contractions of crureus muscle evoked by the branches from the nerves to vastus medialis (CRM) and lateralis (CRL) respectively, the upper records being from the left limb and the lower from the right. The left gracilis nerve had been cross-sutured to the crureus branch from vastus medialis nerve 150 days previously and consequently there is a fast twitch in the upper record of A. The other three contractions were given by the crureus muscle with unmolested innervation. Weight of cat = 3.5 kg. C, D, contractions of left FDL muscle in another animal. One branch only of FDL had been cross-sutured to soleus nerve at operation on the 6-week-old kitten 21 weeks previously. Upper and lower records of C are twitches evoked in the FDL muscle from the soleus and FDL nerves respectively. D shows summation of the slow and fast twitches when partly superimposed. E shows similar summation of wave forms generated electronically. Weight of cat for C, D = 3.4 kg. Note tension scales in grams, giving zero tension. Time scales in 10 msec for D and E.

Investigation of factors modifying the neural influence on muscle

In the preceding paper (Buller et al. 1960) operative isolation of the spinal cord from the remainder of the central nervous system, or, more radically, from the peripheral afferent nervous system as well, was found to result in a failure of the late phase of differentiation of slow muscle.

Apparently under such circumstances there was a serious failure of some neural differentiating influence that was concerned in the slowing of the speed of soleus and crureus contractions. It seems likely that the cross-union experiments give evidence of the operation of the same neural influence. It was therefore of interest to perform the cross-union experiments, when in addition the spinal cord had been operatively isolated.

In four experiments on 4-week-old kittens the standard cross-union was effected between FDL and soleus, with the control operation giving self-regeneration on the other side. One week later the spinal cord from L2 to S2 segmental levels was isolated by transection and all dorsal roots of this stump of cord were transected extradurally, care being taken to preserve the ventral roots uninjured. Thereafter the animals required careful

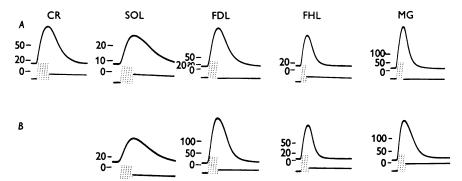


Fig. 10. As in Fig. 1, but with the nerve cross-union and the control operation at age of 28 days. At 35 days the spinal cord was transected above L2 and below S2 and all the dorsal roots from L2 to S2 were severed extradurally on both sides. Final experimental test when animal was 56 days old. The control crureus twitch was recorded only on one side. Weight of kitten = 0.65 kg. Tension scales in grams giving zero tension.

nursing with bladder and bowel emptying twice daily and skin treatment to minimize the development of bed-sores. All survived in good condition sufficiently long for effective regeneration of the severed nerves and the final experimental investigation of the muscle contractions. As illustrated in Fig. 10, there was under such conditions no appreciable change in the speed of muscle contraction as a consequence of the cross-union that had been effected 31 days earlier. The respective contraction times were 54 and 55 msec for the crossed and control soleus muscles, the half-relaxation times being 81 and 82 msec. Similarly the FDL was virtually the same on the two sides, with contraction times of 41 and 43 msec and half-relaxation times of 44 and 40 msec for the crossed and control muscles respectively. There was also no appreciable difference between the repetitive responses on the two sides.

28 PHYSIO. CL

Comparable results were given in the other three experiments, as illustrated by the plotted points of Fig. 11A, B. When compared with the cross-union experiments uncomplicated by the 'isolation' operation (Fig. 3A, B), the FDL contractions on the control side (filled circles in A) were slower, while the control soleus contractions (filled circles in B) at the two longest test intervals were faster. In part the slowing of the FDL contractions relative to the standard age—contraction-time curve (continuous line in Fig. 11A) is attributable to the aftermath of nerve degeneration and regeneration, as is seen in the three control values (filled circles) at 4–6 weeks post-operatively in Fig. 11A. However, there was in

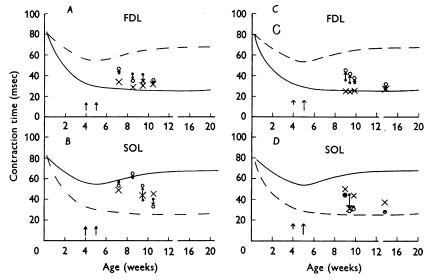


Fig. 11. A, B, plotting as in Fig. 3 of effect of cross-union on contraction times of FDL and soleus muscles, but with in addition the cord isolating operation as in Fig. 10. The oblique crosses show mean values for the contraction times of those fast and slow muscles, respectively, which remained on both sides with unmolested innervation, LG, MG, PL, FHL in A and crureus in B. C, D, as with A, B, control operation at 28 days, but the subsequent operation at 35 days was limited to transection of the spinal cord at L2 level. Again the oblique crosses give mean contraction times for other muscles. In A-D the arrows at 4 weeks show time of nerve cross-union operation, and arrows at 5 weeks the time of the later cord operation. The standard curves for the contraction times are drawn as continuous and broken lines with the same conventions as in Fig. 3.

addition a slowing of the fast muscles with unmolested innervation, as is indicated by the oblique crosses lying above the standard curve in Fig. 11 A, each cross being the average of the contraction times for four fast muscles on each side. Thus it appears that the slowing of the control FDL contraction times in Fig. 11 A was due to the operation of two factors. Cross-innervation by the soleus nerve fibres gave no additional effect,

there being a slight slowing in two experiments and a slight quickening in the other two.

As would be expected, the soleus observations were complicated by another factor. The control observations on the crureus muscle with unmolested innervation (oblique crosses) reveal the same progressive shortening of contraction time that was observed both with soleus and crureus when the isolated cord operation was performed on younger animals (Buller et al. 1960, fig. 9). The contractions of the control soleus muscles with self-regeneration (filled circles) did not differ in any systematic way from these unmolested crureus responses. Thus in Fig. 11 B the operative isolation of the spinal cord provided the over-riding factor determining the speeds of contraction of the soleus muscles. Cross-reinnervation by the FDL nerve caused no demonstrable additional effect, as is indicated by two experiments giving shortening and two lengthening (note arrows).

A similar absence of transformation by the cross-innervation was also shown by the other indicators of muscle speed, half-relaxation and the stimulus intervals for half-tetanic summation. The values for the individual muscles showed changes comparable with those occurring in the contraction times in Fig. 11A, B, there being likewise no significant change in the mean values.

When the initial operative lesion was merely transection of the spinal cord in the upper lumbar region, there was evidence of a less complete deprivation of the neural influence responsible for slowing the soleus and crureus contractions (Buller et al. 1960, figs. 9. 10). It would, therefore, be expected that after such an operation the cross-union experiment would provide evidence of a neural influence on muscle of the same kind as that normally observed, but smaller in amount. The nerve cross-union operation together with the control operation was performed on kittens 4 weeks old and one week later the spinal cord was transected in the upper lumbar region, i.e. this latter operation was performed well before the reinnervation was established. When the muscle contractions were investigated 4 weeks after the initial operation, it was found that there had been a small transformation of the muscles with alien innervation (Fig. 12). For example the FDL muscle with soleus innervation had lengthened contraction times and half-relaxation times, 36 msec lengthened to 45 msec in both cases, while the soleus muscle with FDL innervation had a faster twitch, the halfrelaxation time being shortened from 51 to 35 msec, though the contraction times were both 44.5 msec. The repetitive responses indicated comparable changes, the stimulus interval for half-tetanic summation being lengthened from 33 to 54 msec for FDL and shortened from 62 to 39 msec for soleus. However, as is shown for the plotted points of the

muscle contraction times for the four experiments in Fig. 11C, D, the muscle transformation was on the average much less than half of that normally observed (cf. Fig. 3A, B). This relatively small transformation was also shown by the half-relaxation times and stimulus intervals for half-tetanic summation; the respective lengthenings of the mean values were from 32 to 34 msec and from 35 to 41 msec for the cross-innervated FDL muscles; and the respective mean shortenings were from 43 to 28 msec and from 49 to 36 msec for the cross-innervated soleus muscles.

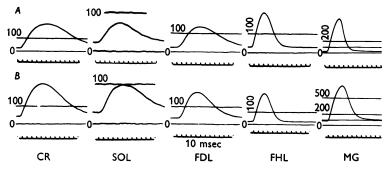


Fig. 12. Series as in Fig. 10, but in which the operation at 35 days was limited to a spinal transection at the L2 level. Measurements from the four experiments of this type are plotted in Fig. $11\,C$, D. Weight of kitten = $0.85\,\mathrm{kg}$. Note tension scales in grams, giving zero tension.

The muscle influence on nerve

The effectiveness of the neural influence on muscle contraction time has obscured a possible alternative relationship—the influence which muscle may exercise on the time characteristics of the innervating motoneurones. So far no attempt has been made to record intracellularly from motoneurones in young kittens, and so to determine whether there is any process of differentiation of motoneurone speeds to match that observed for muscle (Buller et al. 1960); consequently the only experimental evidence relates to motoneurones which on account of nerve cross-union innervate muscles having inappropriate speeds. Since the muscles themselves are so effectively changed by the motoneurones (Figs. 1–6), no large changes could be expected in the reverse direction. Two criteria have been employed in an attempt to detect such a change.

The duration of the after-hyperpolarization following the invasion of a motoneurone by an impulse has uniformly been longer in soleus than gastrocnemius motoneurones (Eccles et al. 1958). Five soleus motoneurones have been recorded from intracellularly after they had innervated the fast muscle, FDL, for over 20 weeks. The contraction times of these FDL muscles were of course considerably slowed as a consequence (cf. Figs. 3, 6),

but the after-hyperpolarization showed no complementary quickening, being still uniformly over 150 msec in duration. Small changes would not be detected by this relatively insensitive test. A more sensitive method of detecting small changes is provided by measurements of conduction velocities of motor axons after cross-union experiments, control measurements being provided by the other limb in which the same nerves were severed and then rejoined to their own peripheral stumps. There was, likewise, no appreciable change (less than 3 % in either direction) in conduction velocity as a result of the cross-union, i.e. within the accuracy of measurement soleus nerve fibres had the same conduction velocity on the two sides, as also did FDL nerve fibres. Thus we may conclude that muscle fibres exert no appreciable influence on the time characteristics of motoneurones that innervate them. It is to be noted that this negative result is obtained for the direction of the modulating influence postulated by Wiersma (1931), Weiss (1950) and Sperry (1950, 1951). It can be concluded that the interaction influencing the time characteristics of motoneurones and muscle fibres occurs very largely, if not exclusively, in the reverse direction, from motoneurones to muscle fibres.

DISCUSSION

In the preceding paper (Buller et al. 1960) it was shown that the operation of isolation of the spinal cord from all incoming impulses caused a failure of the normal process of differentiation of slow muscles, and a simple transection of the spinal cord was rather less effective in the same way. Exactly parallel results have been obtained when the nerve crossunion operation was employed to test for muscle differentiation, cord isolation resulting in the complete failure of nerve cross-union to change muscle speed (Fig. 11A, B), while after cord transection it produced only a small change (Fig. 11C, D). It may be postulated, therefore, that the same neural influences are bringing about the muscle transformation in the normal process of muscle differentiation and in the artificial conditions created by the nerve cross-unions.

The simplest explanation of the influence of motoneurones on muscle speed would be that it is due to the frequency of impulse discharge: the contraction time of a slow muscle is accelerated when subjected to the high-frequency discharges from fast (phasic) motoneurones; while a slowing of contraction time ensues when a fast muscle is subjected to the relatively slow frequencies of discharge from tonic motoneurones. Phasic motoneurones usually discharge at frequencies from 30-60/sec, while with tonic motoneurones the frequency is usually 10-20/sec (Denny-Brown, 1929; Adrian & Umrath, 1929; Granit, Henatsch & Steg, 1956; Granit, Phillips, Skoglund & Steg, 1957).

It would be very difficult technically to arrange artificial stimulation of several weeks duration so that there was a prolonged transposition of these characteristic frequencies of action, independently of nerve cross-union. However, the postulated continuous relationship between frequency of activation and the resultant muscle speed can be tested by eliminating all discharge from motoneurones. This requirement is achieved by the isolated spinal cord operation, as was first demonstrated by Tower (1937 a, b). Under such conditions there is no inflow of impulses into the isolated lumbosacral segments of the spinal cord, and consequently no discharge therefrom, the hind limbs remaining absolutely flaccid. According to the frequency hypothesis it would be predicted that the complete cessation of all discharge would give a more effective slowing of a fast muscle than the low frequency of activation consequent on reinnervation from soleus motoneurones (Fig. 3A). But the reverse is observed: the small lengthening of contraction time (about 5 msec above the standard curve) for the oblique crosses in Fig. 11A contrasts with the much larger lengthening for the soleus-reinnervated FDL muscle in Fig. 3A, C, which was about 20 msec longer on the average. Similarly the small lengthenings of the half-relaxation time and of the stimulus interval for half tetanic summation contrast with the much larger lengthenings in Fig. 6 A, C. The frequency hypothesis fails even more significantly in Fig. 11B (oblique crosses), where cessation of all motoneuronal discharge to crureus muscle caused a considerable quickening of its contraction (contraction times 10-20 msec shorter than for the standard curve), which is the reverse change from the slowing that would be predicted.

An alternative hypothesis would be that muscle speed was controlled by the aggregate number of impulses activating the muscle fibres in a given time, the higher the aggregate the slower the muscle speed; and that the more continuously active tonic motoneurones would actually be delivering a higher aggregate despite their lower frequency. It certainly would be surprising if the sharp differentiation of muscles into fast and slow types (cf. Buller et al. 1960) were effected by such a relatively variable factor as the aggregate number of impulses fired by tonic and phasic motoneurones respectively, particularly when it seems that animals differ so much in their phasic activity. But in addition to this general criticism, the aggregate hypothesis is contra-indicated by the effects of reducing the aggregate to zero. The predicted quickening of slow muscles does indeed then occur (Fig. 11B, oblique crosses), but it certainly is no larger than when slow muscles are reinnervated by phasic motoneurones (Fig. 3B, D). Moreover, the oblique crosses in Fig. 11 A show that with zero activation fast muscles are slower than normal, whereas the aggregate hypothesis would predict the opposite change.

A third hypothesis has been suggested by Mr A. F. Huxley. A fast muscle would give a grossly oscillatory response at the prevalent frequency of stimulation provided by slow motoneurones (cf. Buller $et\ al.$ 1960, Fig. 5C-E, at $13/\mathrm{sec}$), and conceivably this oscillation could effect the gradual transformation to a slow muscle, so alleviating the stress of the oscillatory contraction. When not acted on by this postulated differentiating influence, i.e. with higher frequencies of stimulation or with zero stimulation, all muscle fibres become fast. Since slow motoneurones vary so much in their frequency of discharge, it seems unlikely that the sharp separation which characterizes fast and slow muscles could be produced by such a frequency-dependent influence. This hypothesis has the virtue that it can be tested experimentally: for example, it would predict that the muscles of a spinal animal would be slowed (in contrast to Fig. 9 D, Buller $et\ al.$ 1960) if they were artificially stimulated at the postulated frequency of $10-20/\mathrm{sec}$.

A further possibility is that some specific influence is exerted by the impulses discharged by tonic and phasic motoneurones along their axons. Tonic motoneurones certainly differ in that they have smaller motor axons (Eccles & Sherrington, 1930; Hagbarth & Wohlfart, 1952), which consequently have slower conduction velocities and higher thresholds (Eccles et al. 1958) and give smaller spikes when recorded in a nerve trunk (Granit et al. 1956, 1957). It is not known if this relative difference between the motor axons in the nerve trunks continues down to the fine terminal branches that make the actual contact with the muscle fibres at the motor end-plates, but in any case it seems inconceivable that it could provide the differentiating factor for muscle speed. Furthermore, it does not seem warranted to postulate some difference in the chemical transmitters liberated at the neuromuscular junctions formed by the tonic and phasic motoneurones respectively. Pharmacological investigations have revealed quantitative differences between the neuromuscular junctions to fast and slow muscles (Paton & Zaimis, 1951; Zaimis, 1954; Jewell & Zaimis, 1954; Bowman & Zaimis, 1958), but these slight differences have not shaken the belief that acetylcholine is the transmitter at both types of junction.

Before attempting to define more precisely the nature of the neural influences responsible for muscle speed, it is expedient to consider the possibilities that the influence is exerted through afferent fibres or sympathetic fibres. The latter possibility can virtually be excluded because the sympathetic innervation of the hind-limb muscles would largely be intact in the experiments illustrated in Figs. 10, 11, where the cord section was at the second or third lumbar segment. However, a more crucial test would be provided by cross-union experiments on animals in which the sympathetic innervation of the hind limb had been eliminated by excision of the sympathetic trunk at the appropriate levels. An influence of afferent fibres on muscle is highly improbable because they do not enter into close relationship with the extrafusal fibres of muscle, which are responsible for all the tension developed in muscle

contraction (Kuffler, Hunt & Quilliam, 1951); and no histological changes occur in muscle fibres when the afferent innervation of muscle is degenerated (Tower, 1931). However, evidence for an influence of the afferent innervation is provided by the finding that after months of de-afferentation the contraction of slow muscles was appreciably faster than on the control side, the fast muscles being unchanged (Buller et al. 1960), which is a slight effect of the same kind as with spinal cord transection or isolation. It has been suggested (Buller et al. 1960) that de-afferentation produced this effect by diminishing the activity of the tonic motoneurones. A crucial investigation designed to exclude a direct action of afferent fibres on muscle would be to effect cross-unions on the nerves to muscles de-afferented by appropriate ganglionectomies, but this has not yet been done.

When attempting a more critical evaluation of the evidence relating to neural influences and muscle differentiation, it emerges that a neural influence that slows muscle contraction has been indubitably established, whereas the evidence is less decisive for the existence of an opposite neural influence that quickens muscle contraction. For example, after transection or 'isolation' of the spinal cord in the young kitten, the differentiation of fast muscles did not appreciably differ from normal, whereas there was almost complete failure of the differentiation of slow muscles (Buller et al. 1960). However, when the isolated spinal cord operation was performed on older kittens (5 weeks), the fast muscles were appreciably slower at 7-101 weeks of age (Fig. 11A, oblique crosses), which suggests that there has been defection of a small quickening influence that is normally exerted by fast motoneurones on muscle. The cross-union experiments probably also provide evidence of a fast neural influence that accelerates the soleus muscle contraction, though conceivably this effect could be entirely due to the deprivation of the slow neural influence. Further investigation is required on the time course of the onset of changes in muscle speeds after nerve cross-union.

The changes that nerve cross-unions effect in muscle contraction times are much larger than can be accounted for by changes in conduction velocity and duration of impulses travelling along the muscle fibres from the motor end-plates. For example, the spike potentials of slow muscles are a little longer than those of fast muscles and their propagation velocity is a little slower; but a transformation of a fast muscle in these respects would account at most for a lengthening of contraction time by 3–4 msec (Eccles & O'Connor, 1939). Thus the muscle transformation must occur not only along the whole length of the muscle fibre, but it must also involve the actual contractile mechanism of each element. As yet there has been no histological investigation of the transformed muscles, but a question of great interest concerns the possibility of a transformation of 'Fibrillinstruktur' to 'Felderstruktur' and vice versa, in accordance with the characteristic fibre patterns described by Krüger (1952) for fast and slow muscle.

The muscle transformations brought about by the nerve cross-unions have been assessed by three different measurements for the speed of muscle contraction, the rising time to the summit of a twitch, the half-relaxation time from that summit, and the stimulus interval for a repetitive stimulation that gives a tetanic contraction half way between the twitch and the maximum tetanic contraction. The similar results given by these three criteria indicate that there has been a fairly uniform transformation of all the fibres of a muscle. Since comparable changes have been observed with these three different measurements both after cross-union (Figs. 3, 6) and also with the normal processes of differentiation of slow and fast muscles (Buller et al. 1960, Figs. 3, 4, 6), it can be postulated that the differentiation of slow and fast muscles is brought about by changing the time course of the 'active state' developed in the muscles. Changes in such passive properties as the series-elastic component or in the viscosity of the muscle might cause considerable changes in the time course of a twitch, but not in the summation of tetanic responses at various frequencies. It would be difficult to apply the more precise tests for 'active state' that have been developed by Hill and his collaborators (Hill, 1949, 1953; Jewell & Wilkie, 1958), but investigations of the type described by Macpherson (1953), by Macpherson & Wilkie (1954) and by Ritchie (1954) could be applied in an attempt to provide quantitative measurements of the alterations that nerve cross-unions bring about in the durations of the 'active state'.

It will be convenient now to employ a diagram (Fig. 13) in developing a specific chemical hypothesis of the neural influence exerted by nerve cells on muscle fibres. The postulated neural influence would be exerted by a specific chemical substance that courses along the motor nerve fibres, as shown in Fig. 13, then traverses the neuromuscular junction and finally passes thence along the whole length of the muscle fibres, inducing therein changes that alter the speed of the muscle contraction. There is convincing experimental evidence for a neural influence emanating from tonic motoneurones and slowing muscle contraction. There also may be a different influence emanating from fast motoneurones and quickening muscle contraction, but the evidence for this is much less satisfactory. The hypothesis illustrated in Fig. 13 can account for the absence of neural influence by motoneurones in the 'isolated' and completely quiescent spinal cord (Figs. 10, 11A, B; cf. also Buller et al. 1960) in four different ways: the substance may not be produced in motoneurones or transported thereto in the absence of synaptic bombardment; it may not be able to travel down motor axons in the absence of impulses; it may not be able to cross quiescent neuromuscular junctions; the pumping action of muscle contractions may be required to propel the substance from the motor end-plate region along the whole length of the muscle fibres where it exerts its

transforming action. Experiments are being undertaken to test several of these alternatives. Presumably the mere transection of the spinal cord causes a diminution of the neural influence (Figs. $11\,C,D,\,12$) by the relative depression of cord activity thereby induced.

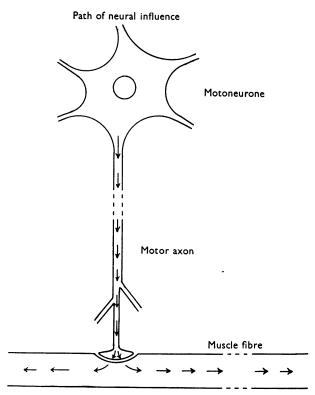


Fig. 13. Diagram to show postulated path for the flow of the slow (and fast) neural influences from the motoneurone down the motor axon and along the muscle fibre.

Since muscles of full-grown cats were transformed just as effectively as those of kittens (Figs. 3, 5, 6), the neural influence must be continuously exerted by nerve cells throughout life. Thus we are introduced to a new concept, that of a continuously operating differentiating principle. Furthermore this principle is exerted by slow motoneurones to slow the contractions of muscles, so appropriately relating their responses to the slow frequencies of discharge of tonic motoneurones. It is therefore of significance in improving the efficiency of operation of tonic motor units. The question thus arises: Are influences of a comparable kind exerted more generally, so enhancing the efficiency of other functional connexions between cells, for example not only from neurone to muscle fibre, but also from neurone to neurone?

Consideration should be given to the possible relationship of these neural influences on muscle contraction time to the 'trophic' influences which have been regarded as a separate phenomenon from the activation of muscle by nerve impulses (Tower, 1937 a, b; 1939; Eccles, 1941 a, b; Luco & Eyzaguirre, 1955). For example the supersensitivity to acetylcholine, the spontaneous fibrillation and certain histological changes occur when the motor nerve is severed, but not when muscle is simply deprived of nerve impulses. It has been found that the first two effects take longer to develop the more central the nerve section (Luco & Eyzaguirre, 1955). Consequently it has been postulated that there is some 'trophic' influence in the motor axon which slowly spreads along it with a speed at most of 2 mm/hr. Axelsson & Thesleff (1959) have shown that after denervation of mammalian muscles sensitization to acetylcholine develops uniformly along the whole length of the muscle fibres, there being no special change at the end-plate region. Apparently this 'trophic' influence of the motor axon operates to restrict the acetylcholine sensitivity to the end-plate region, an effect which is particularly well illustrated by the spreading sensitivity that develops only at the denervated end-plates on muscle fibres with multiple innervation (Miledi, 1959). Moreover, this action is independent of nerve impulses, for the sensitization does not occur in disused muscle. This trophic influence on the surface membrane of the muscle fibre is presumably distinct from the postulated fast and slow neural influences on the contractile mechanism of the muscle fibre.

SUMMARY

- 1. Nerve cross-union experiments have been employed in the cat hind limb in order to discover if motoneurones determine the speed of muscles, or if the effective influence is in the reverse direction.
- 2. When a nerve from fast or phasic motoneurones has been made to innervate a slow muscle, the muscle is transformed to a fast muscle, even in the adult; likewise slow or tonic motoneurones convert fast muscles to slow.
- 3. The transformation of muscle speed is shown not only by the time course of the rising and falling phases of the twitch contraction, but also by the summation of tetanic contractions at various frequencies.
- 4. The slow or fast muscle with alien innervation has no detectable influence in the reverse direction, i.e. on the motoneurones. The conduction velocity of the motor axons and the time course of motoneural after-hyperpolarization are both unchanged.
- 5. The muscle transformation falls short of a complete change to the slow or fast type.

- 6. Isolation of the lumbosacral spinal cord from all incoming impulses, with consequent silence of motoneurones, causes a failure of all transformation by cross-union. Mere transection of the spinal cord greatly reduces the transformation.
- 7. It is concluded that the neural influence on muscle speed is not exerted by nerve impulses as such. It is postulated that a substance passes down the axons of slow motoneurones, crosses the neuromuscular junctions and traverses the muscle fibres, transforming them into slow contracting units and maintaining them so. Possibly there is also a substance from fast motoneurones that acts via a comparable pathway to accelerate muscle contraction.

REFERENCES

- Adrian, E. D. & Umrath, K. (1929). The impulse discharge from the Pacinian corpuscle. J. Physiol. 68, 139-154.
- Axelsson, J. & Thesleff, S. (1959). A study of supersensitivity in denervated mammalian skeletal muscle. J. Physiol. 147, 178–193.
- Bowman, W. C. & Zaimis, E. (1958). The effects of adrenaline, noradrenaline and isoprenaline on skeletal muscle contractions in the cat. J. Physiol. 144, 92–107.
- Brown, G. L. (1937). Action potentials of normal mammalian muscle. Effects of acetylcholine and eserine. J. Physiol. 89, 220–237.
- Brown, G. L., Dale, H. H. & Feldberg, W. (1936). Reactions of the normal mammalian muscle to acetylcholine and to eserine. J. Physiol. 87, 394—424.
- Buller, A. J., Eccles, J. C. & Eccles, R. M. (1958). Controlled differentiation of muscle. J. Physiol. 143, 23-24 P.
- Buller, A. J., Eccles, J. C. & Eccles, R. M. (1960). Differentiation of fast and slow muscles in the cat hind limb. J. Physiol. 150, 399-416.
- COOPER, S. & ECCLES, J. C. (1930). The isometric responses of mammalian muscles. J. Physiol. 69, 377-385.
- Denny-Brown, D. (1929). On the nature of postural reflexes. *Proc. Roy. Soc.* B, 104, 253-301.
- Eccles, J. C. (1941a). Changes in muscle produced by nerve degeneration. *Med. J. Aust.* 1, 573-575.
- Eccles, J. C. (1941b). Disuse atrophy of skeletal muscle. Med. J. Aust. 2, 160-164.
- Eccles, J. C., Eccles, R. M. & Lundberg, A. (1958). The action potentials of the alpha motoneurones supplying fast and slow muscles. J. Physiol. 142, 275-291.
- ECCLES, J. C. & O'CONNOR, W. J. (1938). Responses evoked by a nerve volley in mammalian striated muscle. J. Physiol. 94, 7-8 P.
- ECCLES, J. C. & O'CONNOR, W. J. (1939). Responses which nerve impulses evoke in mammalian striated muscles. J. Physiol. 97, 44-102.
- Eccles, J. C. & Sherrington, C. S. (1930). Numbers and contraction-values of individual motor-units examined in some muscles of the limb. *Proc. Roy. Soc.* B, **106**, 326–357.
- Feng, T. P., Tsung-Han Li & Yen-Chieh Ting (1939). Studies on the neuromuscular junction. XII. Repetitive discharges and inhibitory after-effect in post-tetanically facilitated responses of cat muscles to single nerve volleys. *Chin. J. Physiol.* 14, 55–80.
- Granit, R., Henatsch, H. D. & Steg, G. (1956). Tonic and phasic ventral horn cells differentiated by post-tetanic potentiation in cat extensors. *Acta physiol. scand.* 37, 114-126.
- Granit, R., Phillips, C. G., Skoglund, S. & Steg, G. (1957). Differentiation of tonic from phasic alpha ventral horn cells by stretch, pinna and crossed extensor reflexes. J. Neurophysiol. 20, 470-481.
- GUTH, L. (1956). Regeneration in the mammalian peripheral nervous system. *Physiol. Rev.* 36, 441–478.
- GUTMANN, E. (1945). The reinnervation of muscle by sensory fibres. J. Anat. 79, 1-8.

- GUTMANN, E. (1958). Die funktionelle Regeneration der peripheren Nerven. Berlin: Akademie-Verlag.
- HAGBARTH, K. E. & WOHLFART, G. (1952). The number of muscle-spindles in certain muscles in cat in relation to the composition of the muscle nerves. *Acta anatomica*, 15, 85–104.
- HILL, A. V. (1949). The abrupt transition from rest to activity in muscle. Proc. Roy. Soc. B, 136, 399-420.
- HILL, A. V. (1953). The mechanics of active muscle. Proc. Roy. Soc. B, 141, 104-117.
- Jewell, B. R. & Wilkie, D. R. (1958). An analysis of the mechanical components in frog's striated muscle. J. Physiol. 143, 515-540.
- Jewell, P. A. & Zaimis, E. J. (1954). A differentiation between red and white muscle in a cat based on responses to neuromuscular blocking agents. J. Physiol. 124, 417–428.
- KRÜGER, P. (1952). Tetanus und Tonus der quergestreiften Skelettmuskeln der Wirbeltiere und des Menschen. Leipzig Akad. Verlagsgesellschaft: Geest und Poitig K-G.
- Kuffler, S. W., Hunt, C. C. & Quilliam, J. P. (1951). Function of meduliated small-nerve fibres in mammalian ventral roots: efferent muscle spindle innervation. *J. Neurophysiol.* 14, 29–54.
- Luco, J. V. & Eyzaguirre, C. (1955). Fibrillation and hypersensitivity to ACh in denervated muscle: effect of length of degenerating nerve fibers. J. Neurophysiol. 18, 65-73.
- MACPHERSON, L. (1953). A method of determining the force-velocity relation of muscle from two isometric contractions. J. Physiol. 122, 172-177.
- MACPHERSON, L. & WILKIE, D. R. (1954). The duration of the active state in a muscle twitch. J. Physiol. 124, 292-299.
- MILEDI, R. (1959). Acetylcholine sensitivity of partially denervated frog muscle fibres. J. Physiol. 147, 45 P.
- Paton, W. D. M. & Zaimis, E. J. (1951). The actions of p-tubocurarine and of decamethonium on respiratory and other muscles in the cat. J. Physiol. 112, 311-331.
- RITCHIE, J. M. (1954). The duration of the plateau of full activity in frog muscle. J. Physiol. 124, 605-612.
- Sperry, R. W. (1950). Neuronal Specificity. In *Genetic Neurology*, ed. Weiss, P., pp. 232–239. University of Chicago Press.
- Sperry, R. W. (1951). Mechanisms of neural maturation. In *Handbook of Experimental Psychology*, ed. Stevens, S. S. New York: John Wiley and Sons.
- Tower, S. S. (1931). A search for trophic influence of posterior spinal roots on skeletal muscle, with a note on the nerve fibers found in the proximal stumps of the roots after excision of the root ganglia. *Brain*, **54**, 99-110.
- Tower, S. S. (1937a). Function and structure in the chronically isolated lumbo-sacral spinal cord of the dog. J. comp. Neurol. 67, 109-131.
- Tower, S. S. (1937b). Trophic control of non-nervous tissues by the nervous system. A study of muscle and bone innervated from an isolated and quiescent region of spinal cord. J. comp. Neurol. 67, 241-267.
- Tower, S. S. (1939). The reaction of muscle to denervation. Physiol. Rev. 19, 1-48.
- Weiss, P. (1950). An introduction to genetic neurology. In *Genetic Neurology*, ed. Weiss, P. University of Chicago Press.
- Weiss, P. & Edds, M. V. (1945). Sensory motor nerve crosses in the rat. J. Neurophysiol. 8, 173-194.
- WIERSMA, C. A. G. (1931). An experiment on the 'Resonance Theory' of muscular activity. Arch. neer. Physiol. 16, 337-345.
- ZAIMIS, E. J. (1954). Transmission and block at the motor end-plate and in autonomic ganglia. *Pharmacol. Rev.* 6, 53-57.